# Development of a Lactate Biosensor for Monitoring the Physical Fitness of Astronauts

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## Introduction

**Lactate used and levels**

- Lactate is a metabolite formed from oxidation of pyruvate in muscles and liver
- The normal range of lactate in blood is 0.5-2.5 mM [1]

**Elevate lactate concentration can indicate** [2]

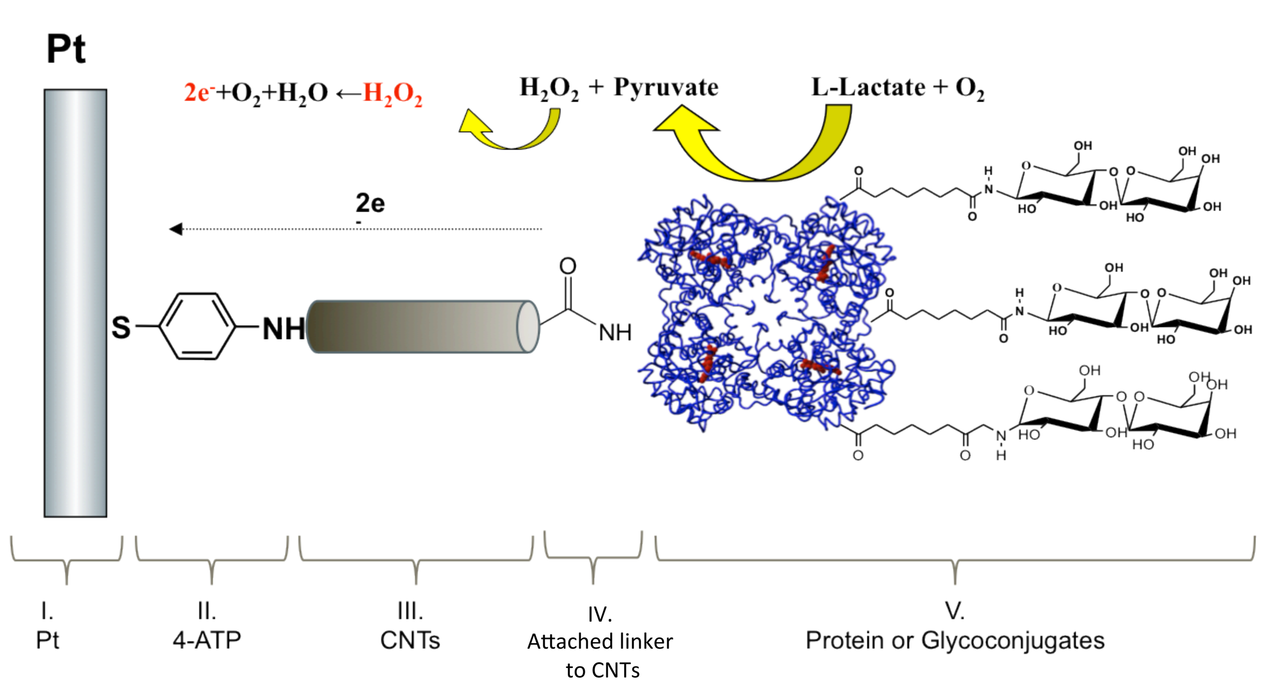
- Multiple organ failure (e.g. liver, kidney, heart)
- Septic shock
- Contribute to lactic acidosis in tissues and blood

**Lactate biosensor useful** [3]

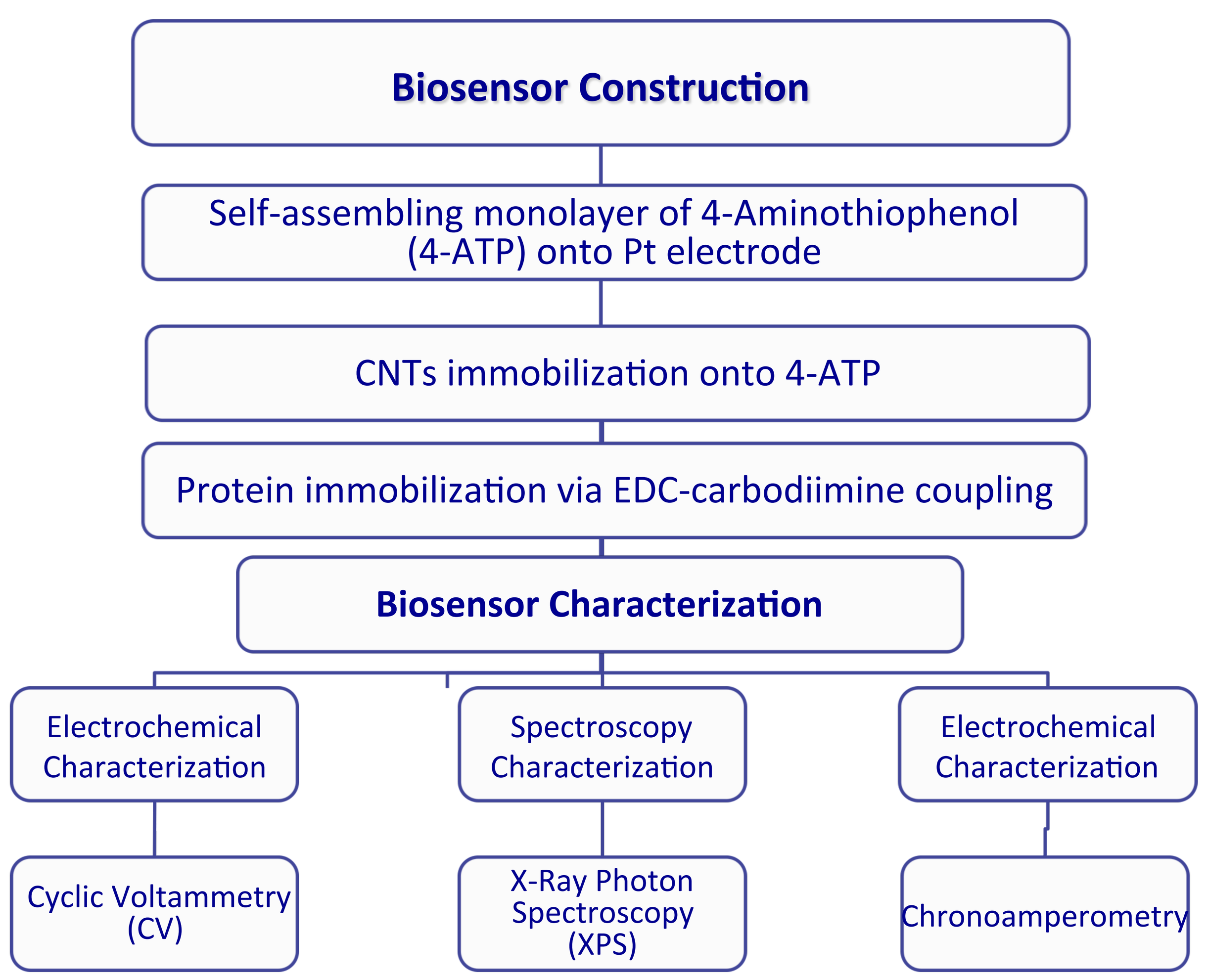
- Diagnosis and treatment of medical conditions (e.g. cystic fibrosis , septic shock)
- Relevant in sport medicine for estimation of the physiological condition of athletes

***The goal*** is to develop an enzyme-based biosensor for monitoring lactate concentration in blood. The model protein for this sensor is lactate oxidase (LOx).

***This biosensor is an excellent match with NASA necessities to monitor astronaut's health during space missions since astronauts' muscles and bones get weakness due to gravity reduction [4].***



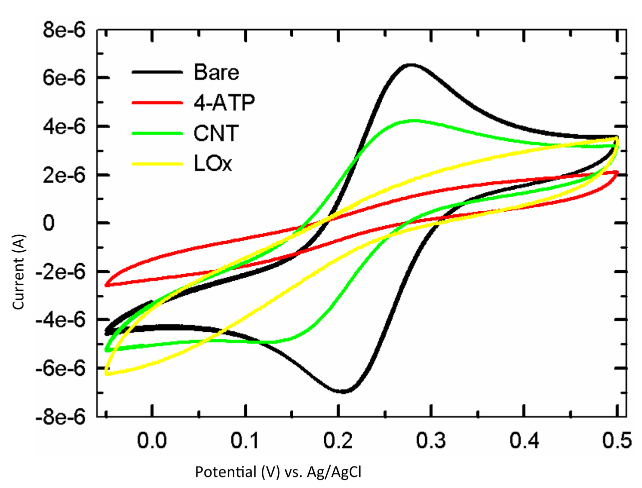

## Methodology and Characterization




## Results

### Electrochemical Characterization

**Figure 1: Cyclic voltammogram of the electrode Pt-4ATP-CNT-Enz in 1.0 mM $K_3Fe(CN)_6$ / $K_4Fe(CN)_6$ in PBS at pH 7.0.**




### Spectroscopy Characterization

**Figure 2: XPS at each step of the biosensor construction.**

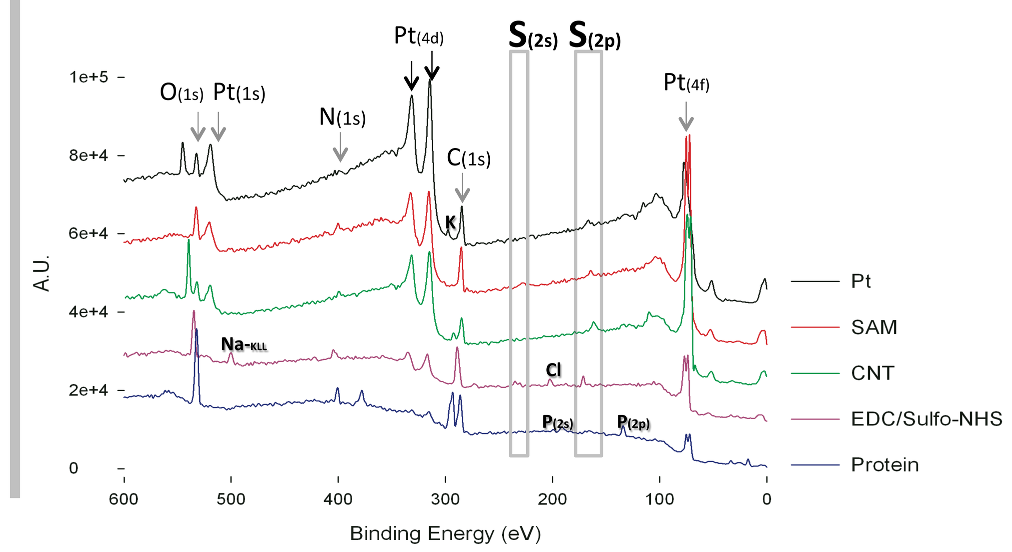



**Figure 3: XPS at each step of the biosensor construction. Zoom in the sulfur region (250-150 eV).**

| Group | 2p line (eV) |
|---|---|
| Sulfure (S) | 164 |
| Cystein (-SH) | 163.2 |
| Sulfite (-$SO_3$=) | 165.6-167 |

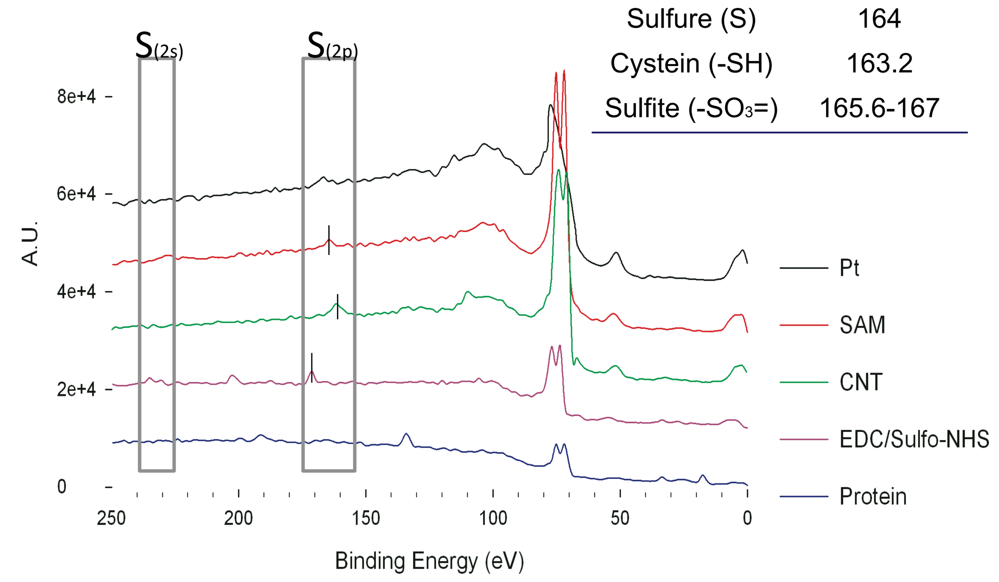
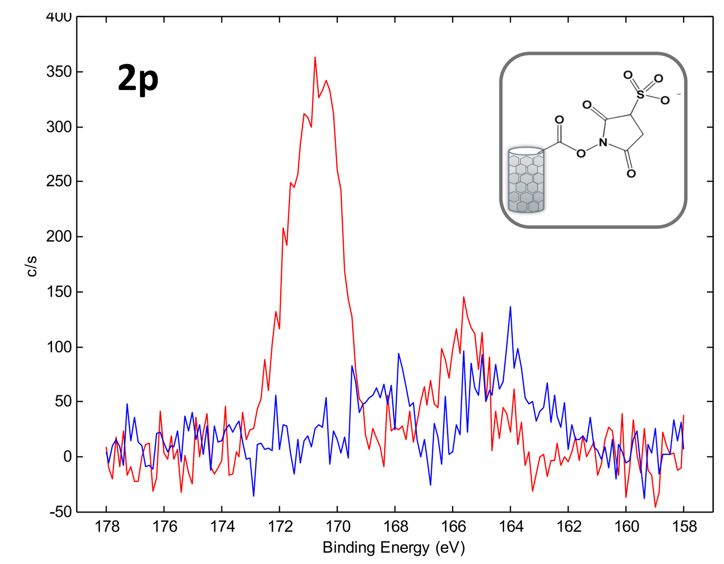



**Figure 4: High Resolution-XPS in the sulfur region.** (red line: sensor with linker, blue line: sensor with protein)




### Electrochemical Characterization

**Figure 5: Amperometric response of the LOx biosensor obtained after the successive addition of 100uL of 0.1M lactate (0.65V vs. Ag/AgCl.)**

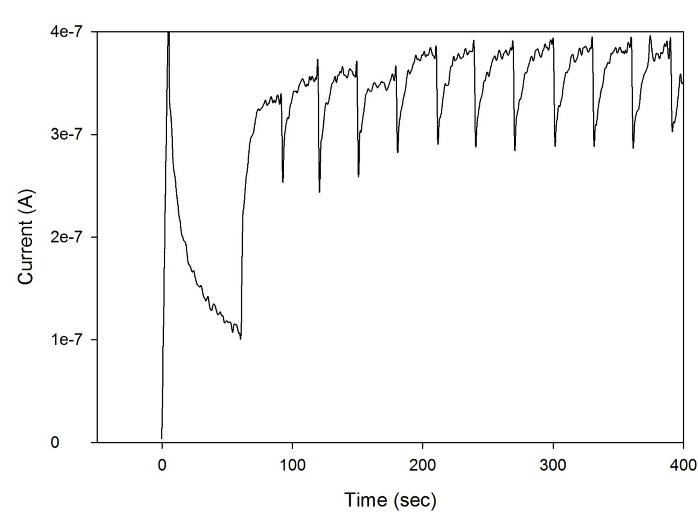
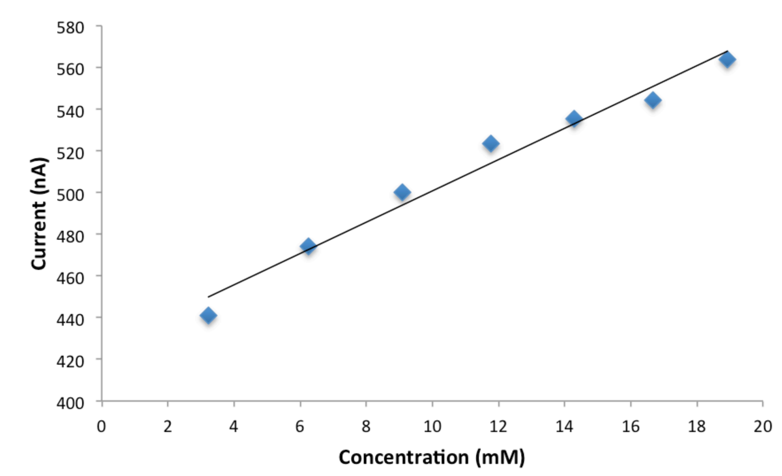



**Figure 6: Calibration curve of the biosensor**




| Electrode | Sensitivity | Detection Limits |
|---|---|---|
| Pt-SAM-CNT-LOx | 8.7± 0.2 nA/mM | 0.44 ± 0.01 mM |

**Acknowledgement**

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## Discussion

- In figure 1, the bare electrode voltammogram shows a cathodic and anodic peak which correspond to a reversible behavior of the electroactive species ($K_3Fe(CN)_6$/$K_4FE(CN)_6$ in solution. After 24 hrs of 4-ATP immobilization, the redox peak of the electroactive species diminishes due to the blocking effect of the monolayer, which does not allow the species in solution to reach the electrode surface. After 12 hrs of covalent immobilization of CNT the current increased since CNTs help the electron transport of the electrospecies. A slight decrease in the current is observed after LOx immobilization since is blocking, in some extent the arriving of the electroactive species to the electrode. This experiment suggests that the biosensor assembly was done properly.

- XPS experiments (Figure 2-4) were used to confirm covalent immobilization of the protein onto the CNT electrode thru the covalent bond between the linker Sulfo-NHS/EDC of the CNT and a protein amine. The linker attached to the CNT form an ester-sulfite leaving group that can be monitored by XPS. In figure 2 and 3, each line in the spectra correspond to the XPS analysis in each of the steps (bare electrode, SAM, CNT and protein immobilization) of the biosensor construction. The pink line corresponding to the EDC/Sulfo-NHS linker shows a peak in 170 ev, which corresponds to the presence of a sulfite (-$SO_3^=$) from the linker. After adding the protein to the electrode (blue line), the peak corresponding to the sulfur group disappears indicating that the CNT forms a covalent bond with the protein. This is confirm in the XPS high resolution spectra in figure 4.

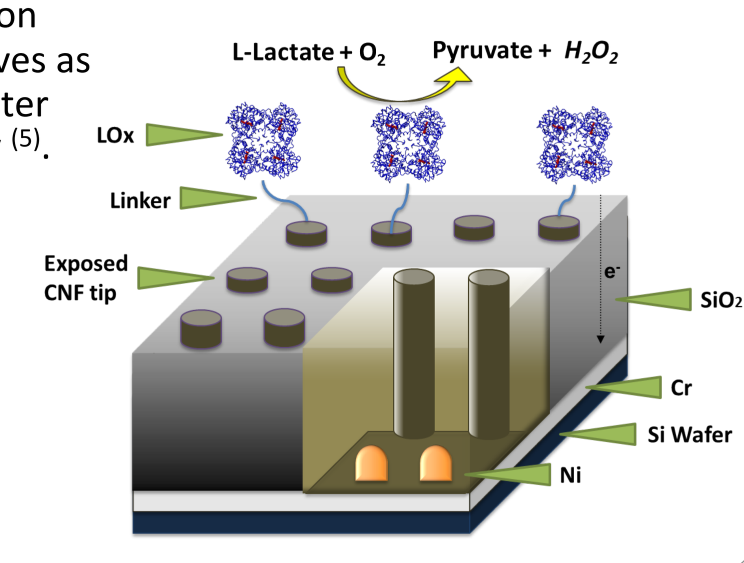
- Figure 5 shows the amperometric response of the LOx biosensor. The biosensor exhibit a fast and sensitive response to changes in lactate concentration. Figure 6 illustrate the calibration curve of LOx biosensor. The current increased linearly with increased in the lactic acid concentration and saturated at a lactate concentration of 15 mM. The calibration curve exhibit a linear response in the range from 3 mM to 12 mM with a sensitivity 8.7± 0.2 nA/mM. The detection limit is is of 0.44 ± 0.01 mM.

## Future Work: Testment

**LOx Biosensor Chip**

A single nanochip contains many carbon nanofibers in which each of one behaves as a single nanoelectrode, presenting faster time response and superior sensitivity [5].

- CNF electrode:
  - ✓ Highest sensitivity
  - ✓ Robust
  - ✓ Real size biosensor
  - ✓ Protects the CNFs
  - ✓ Durable and reusable




**References**

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